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CIVIL ENGINEERING

A study on microbial self-healing concrete using expanded perlite

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Abstract. The increasing concern for the safety and sustainability of structures is calling for the development of smart self-healing materials and preventive repair methods. This research is carried out to investigate the extent of self-healing in normal-strength concrete by using *Sporosarcina aquimarina* – NCCP-2716 immobilized in expanded perlite (EP) as the carrier. The efficacy of crack-healing was also tested using two alternative self-healing techniques, i.e. expanded perlite (EP) concrete and direct introduction of bacteria in concrete. A bacterial solution was embedded in EP and calcium lactate pentahydrate was added as the nutrient. Experiments revealed that specimens containing EP-immobilized bacteria had the most effective crack-healing. After 28 days of healing, the values of completely healed crack widths were up to 0.78 mm, which is higher than the 0.5 mm value for specimens with the direct addition of bacteria. The specimen showed a significant self-healing phenomenon caused by substantial calcite precipitation by bacteria. The induced cracks were observed to be repaired autonomously by the calcite produced by the bacteria without any adverse effect on strength. The results of this research could provide a scientific foundation for the use of expanded perlite as a novel microbe carrier and *Sporosarcina aquimarina* as a potential microbe in bacteria-based self-healing concrete.

Key words: concrete; self-healing; bacteria; expanded perlite; crack detection.

1. INTRODUCTION

Concrete is a popular construction material due to its low cost, durability, strength, and ease of handling [1–3]. Despite remarkable improvements and studies in concrete technology that have made concrete design less porous, the risk of small cracks (0.3 mm) in concrete affecting its strength has remained unchanged. These cracks adversely affect the durability and strength of concrete [4–6]. Furthermore, when compared to conventional strength concrete, brittleness, and early-age cracking are more prominent in recently created high-performance concretes [7]. Although such cracks do not always endanger a structure, they do damage its performance, hasten its decay, harm its sustainability, and shorten its service life [8]. When concrete cracks form a network, permeability is dramatically increased. As a result, the structure is vulnerable to aggressive elements in the environment [9]. Microcracks offer pathways for harmful elements from the environment to enter the steel reinforcement, causing corrosion [10, 11]. As a result, aging and shrinkage-induced durability cracks must be repaired to restore the mechanical and physical qualities of concrete. The development of numerous passive crack treatment procedures has resulted from the necessity to heal cracks.

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Concrete has the capability of self-healing cracks. Self-healing concrete technologies are being employed around the world as a way to improve the durability of concrete [12]. The phenomena of crack healing have been known for many years, and various types of research have been done to investigate it [13]. Self-healing technologies lessen the need for manual labor in structure maintenance and restoration. It reduces the usage of environmentally unfavorable and costly materials that are usually used for concrete restoration, preserving the environment and lowering total structural costs.

Various researchers focus on self-healing concrete and reported a positive response [14]. A researcher [15] used the technique of microbial-induced calcite precipitation (MICP) to mend cracks in mortar specimens. 50 mm × 10 mm cylindrical discs were cracked and then 21 cycles of MICP treatment were applied, with each cracked sample soaking for 2 hours in a 60 mL bacterium solution. In each cycle, the specimen was allowed to drain for 5 minutes to remove the bacterium solution before being immersed in 4 L of urea-$CaCl_2$ solution for roughly 22 hours and then drained for 5 minutes. As a result, $CaCO_3$ was deposited on the damaged surfaces. MICP-treated specimens had tensile strengths ranging from 32 to 386 kPa, while the specimens that were water-treated were too weak to be evaluated. Water permeability studies carried out after 0, 7, 14, and 21 cycles of MICP treatment revealed a considerable decrease in permeability. Even after 7 MICP cycles, little cracks (less than 0.52 mm) were repaired, whereas cracks with a width of up to 1.62 mm were repaired after 21 MICP cycles [15]. Compressive strength, water absorption, self-healing efficiency, surface pore healing, and water penetration depth were evaluated in mortar specimens with different concentrations of microorganisms. Mortar specimens with 105 cells/ml were the most effective in terms of strength augmentation, whereas specimens with 107 cells/ml showed the most precipitation and, as a result, the most crack repair and surface pore healing [16]. Immobilized biochar was used by the study [17] to immobilize *B. sphaericus* in concrete for fracture repair. Concrete also included polypropylene fibers and superabsorbent polymers. The specimens were pre-cracked at 50% and 70% flexural strength, respectively. When compared to other combinations, biochar-immobilized bacteria with polypropylene fibers provided the best recovery of concrete compressive and flexural strength. A study concluded that expanded perlite was discovered to have a beneficial effect on the ability of concrete cracks to repair [18]. A researcher [19] stated that EP particles wrapped with different types of material had their bulk density and water absorptions evaluated using the usual method for lightweight aggregates. The EP bulk density improved significantly after it was wrapped, and the increased carrier density aided in the equal placement of the carrier material in the concrete.

Brief literature shows that different researchers used different techniques for self-healing concrete. However, the knowledge is scattered, and the reader feels difficulty to extract the most efficient techniques. Therefore, a compressive study is required on the comparative study of different techniques used for self-healing. This research investigates the use of species of bacteria for self-healing and also its immobilization by using expanded perlite. The primary objective of this study is to investigate the use of bacterial spores as self-healing agents in concrete. Secondly, to study the effectiveness of expanded perlite as immobilizing technique, and finally the efficiency of *Sporosarcina aquimarina* as a self-healing agent with calcium lactate as the nutrient. Experiments revealed that specimens containing EP-immobilized bacteria had the most effective crack-healing.

2. EXPERIMENTAL SETUP AND METHODOLOGY

2.1. Materials

2.1.1. Bacteria

2.1.1.1. Sporosarcina aquimarina

Sporosarcina aquimarina is a spore-forming, anaerobic, rod-shaped bacterium that has been cultured from seawater. It is around 0.9–1.2 µm broad and 2.0–3.5 µm long [20]. It grows in the presence of 13% NaCl. It does not show growth in an environment containing more than 14% NaCl. Temperatures of 25 degrees Celsius are optimum for growth. The pH range for optimal growth is 6.5–7.0. Growth is slowed below pH 5.0 [21].

Sporosarcina aquimarina has a biosafety grade of 1 (based on the Danger Group Classifications of the Public Health Agency of Canada); hence, it is safe and represents a low risk to laboratory workers and the environment [22]. Figure 1 depicts *Sporosarcina aquimarina* cells used in this study.

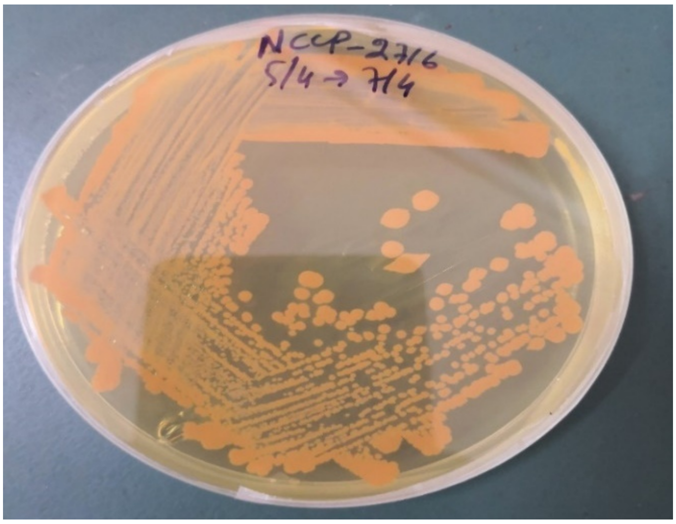

Fig. 1. *Sporosarcina aquimarina* cells

The McFarland standard is a set of standards with varying opacities that are used to assess the density of the bacterial solution. It is frequently used as a standard in a variety of microbiological procedures. A suspension consisting of barium sulphate has a tube number assigned to it. Suspensions of various opacities are given different numbers. Each standard denotes a particular opacity that corresponds to the opacity of bacterial suspensions in actual applications. The aforementioned standard numbers are connected to concentrations and optical densities as reported in Table 1 to determine bacterial concentration.

Table 1
McFarland turbidity standards

Standard	Bacterial concentration (10^6 m/L)	Optical density (550 nm)
0.5	150	0.125
1	300	0.25
2	600	0.5
3	900	0.75
4	1200	1
5	1500	1.25

2.1.1.2. Perlite

Perlite is a volcanic-derived aluminosilicate. It expands to create white, lightweight aggregates (weighing only 0.13 g/cm^3) with a tight cellular structure when crushed and heated fast to 1000°C. Perlite has a density of 128 kg/m^3 (8 lb/ft^3) and is available in a variety of particle sizes. Water is held three to four times its weight due to its tight cellular structure, but only on the surface of the aggregates or in the pore spaces between them. The amount of water available in perlite is determined by the material grade. Coarse perlite, on the other hand, contains very little water. Perlite has almost minimal cation exchange capacity and little buffering capacity, and has a pH of 7.0–7.5, making it neutral. It is mostly made up of silicon dioxide (73%) and aluminum oxide (13%).

2.1.2. Cement

Normal setting time ordinary Portland cement with a density of 385 kg/m^3 was used as a binder in this study. Furthermore, the chemical composition of the cement used in this study is displayed in Table 2.

Table 2
Chemical composition of cement

Compound	Percentage (%)
SiO_2	21
Al_2O_3	5.04
Fe_2O_3	3.24
Al_2O_3	5.04
Fe_2O_3	3.24
CaO	61.7
MgO	2.56
SO_3	1.51
Free lime	0.98

2.1.3. Aggregates

Locally available natural river sand was used as fine aggregate in this study. Normal-weight locally available crush stone was used as a coarse aggregate in this study. Both fine and coarse aggregate keep constant throughout the study. Furthermore, Table 3 depicts the physical aspects of aggregates used in this study.

Table 3
Aggregate properties

Property name	Results	
	Fine	Coarse
Specific gravity	2.64	2.64
Absorption (%)	1.42	0.88
Density (kg/m^3)	850	937
Fineness modulus	3.01	–

2.1.4. Admixture

Admixture (SikaPlast – 512 PK) was used as a plasticizer in this study to increase workability in the mix. Furthermore, the physical aspects of the plasticizer used in this study are presented in Table 4.

Table 4
Properties of superplasticizer

Property name	Results
Appearance	Light brown liquid
Specific gravity	1.150 at 20°C
Density at 25°C	1.05–1.06 kg/lit
PH	5–9

2.2. Preparation of bacterial solution

Growth of bacteria (NCCP-2716 514 714) [*Sporosarcina aquimarina*] culture was achieved in National Agriculture Research Centre (NARC) Islamabad. In the first step, TSB (Tryptone Soya Broth) was mixed with distilled water in 5 flasks and placed in Autoclave (15 psi pressure, 20 minutes, 121°C temperature) for sterilization. Then 5 different salts were mixed in each flask and inoculation was done with a sterilized inoculation needle in a laminar flow clean bench. Figure 2 depicts the preparation of the growth medium. Then the 5 flasks were sealed with corks as shown in Fig. 3 and placed in a shaking incubator. Spores were finally formed. The transparent solution turned turbid which is a clear indication of spore formation. The turbid solution was poured into centrifuge tubes and the tubes were placed in a centrifuge machine that has a capacity of 6 tubes (at max).

After the centrifugation process, the spores got separated from the broth solution and the broth solution again became turbid. The vortex machine vigorously shakes the spores to make a homogenous solution. Now we use a spectrophotometer to dilute the solution to a 0.5 value. Finally, at 0.5 value of absorption, the solution ready to be added to concrete was made.

Fig. 2. Preparation of growth medium (a) TSB solution, (b) autoclave, and (c) Tryptone Soya Broth

Fig. 3. (a) Different salts were mixed in each flask, (b) spores, and (c) prepared bacteria

2.3. Testing setup and mix proportions

The compressive strength of the concrete as per ASTM [23] was detected through the cylindrical sample of standard size, 150 mm in diameter and 300 mm in length. To ensure that crack filling occurs, the cylinders and cubes were cracked and exposed to a submerged water-cured healing environment. To check the recovery in compressive strength, visible cracks were developed on the surface by applying 75–80% compressive load on the reference sample. Then the specimens were subjected to a healing environment to evaluate their compressive strength.

Cracks were visually inspected using a crack detection microscope as shown in Fig. 4 which has a scale with a 0.02 mm precision. The samples were pre-cracked until they developed cracks of varying diameters. Samples were classified as 7 days pre-cracked or 28 days pre-cracked to identify the age at which cracks formed. The initial widths of several cracks were measured and documented. During the curing procedure, both types of samples were completely submerged. The samples were removed after 14 and 28 days of curing and the crack widths were measured once more. The difference in millimeters between the original and final widths was used to indicate healing.

Fig. 4. Crack detection microscope

Before the mixing process started, the required quantity of concrete ingredients was weighed by the system of weighing. The rate of the mixer is kept constant at 30 rev/min for the blending of ingredients. Each ingredient was dry blended with the essential amount of OPC, and water was inserted over time (75% of the water was added first, and then the 25% of water containing mixed superplasticizer in the wet mixing process), and blending was performed around 10 minutes for all batches. Expanded perlite was soaked in a bacterial solution for 10 minutes before being added to the concrete mix. An additional 15% solution was added to ensure thorough soaking of EP and to compensate for any losses. The water-to-cement ratio was set at 0.45. Admixture (SikaPlast-512 PK) was used as a plasticizer to increase workability in the mix. The mixture that did not contain expanded perlite or bacteria was used as a control mix from which other mixes were compared. The mineral precursor, calcium lactate pentahydrate, was introduced during the dry mixing process. The amount of calcium lactate pentahydrate was kept constant for all four batches. Four mixed proportions with different techniques were prepared. Furthermore, the quantification of materials was displayed in Table 5.

Table 5
Quantifications of materials

Mix ID	Control	EP	Direct bacteria	Bacteria + EP
Cement (kg/m^3)	385	385	385	385
FA (kg/m^3)	758	758	758	758
CA (kg/m^3)	1018	1018	1018	1018
W/C	0.45	0.45	0.45	0.45
Plasticizer (%)	1.2	1.2	1.2	1.2
Bacteria solution (L/m^3)	–	–	13	13
Calcium lactate (%)	4	4	4	4
Expanded perlite (g)	57.9	57.9	57.9	57.9

3. RESULTS AND DISCUSSION

3.1. Compressive strength

The compressive strength with and without Expanded Perlite (EP) reinforced microbial concrete at 28 days is shown in Fig. 5. Type of bacteria, bacterial percentage, strategy, and incubation period are the primary factors of compressive strength [24, 25]. It can be observed that the compressive strength of concrete slightly decreased with the addition of EP i-e 7.6% less than from the reference mix (concrete without EP). Similarly, a study also claimed that the compressive strength values decline with the addition of EP [26]. The decrease in compressive strength can be retributed due to EP weaker strength and porosity which ultimately decreased compression strength. The findings of an additional study indicated that when the EP aggregate content was raised, the overall porosity of the concrete also increased [27] due to the porous nature of EP. The increase in porosity results in decreases in strength properties [28–30]. However, in the case of direct bacteria addition, a slightly increased compressive strength was observed which was 6.5% more than from reference blends. A study [31] observed that the bacteria can repair early cracks in the mortar. According to research, concrete compressive strength is improved when bacteria of the *Sporosarcina pasteurii* species are present at quantities of 10^5 cells per milliliter [32]. More bacteria added to concrete has a detrimental impact on its quality [33]. This is due to the fact that there is intense nutritional competition between cells when cell density rises. In comparison to normal concrete, the compressive strength is reduced by around 10% when the concentration of bacteria cells exceeds the optimal threshold [34]. However, in the case of combined addition (EP and bacteria), the compressive strength is approximately equal to the reference concrete. It is because EP decreased compressive strength and bacteria improved compressive strength. Therefore, a combination of EP and bacteria can be used without any adverse effect on compressive strength.

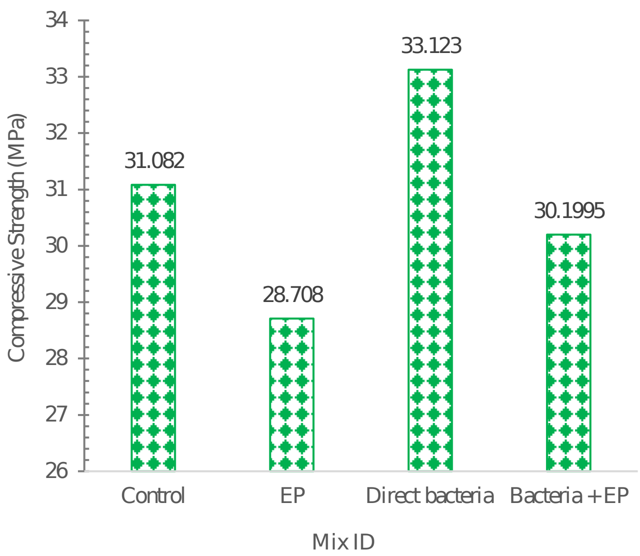

Fig. 5. Compressive strength

3.2. Visual observation of self-healing

Concrete specimens cracked after 28 days of casting were observed visually for observation of crack filling. Figure 6 shows the healing crack width. Because the width of cracks was not possible to be kept in a certain control while the load was being applied, the cracks appeared to be of different widths. $CaCO_3$ generation on the surface of the fractures was visually recognized and quantified using digital image processing after the majority of the fractured areas healed after 28 days.

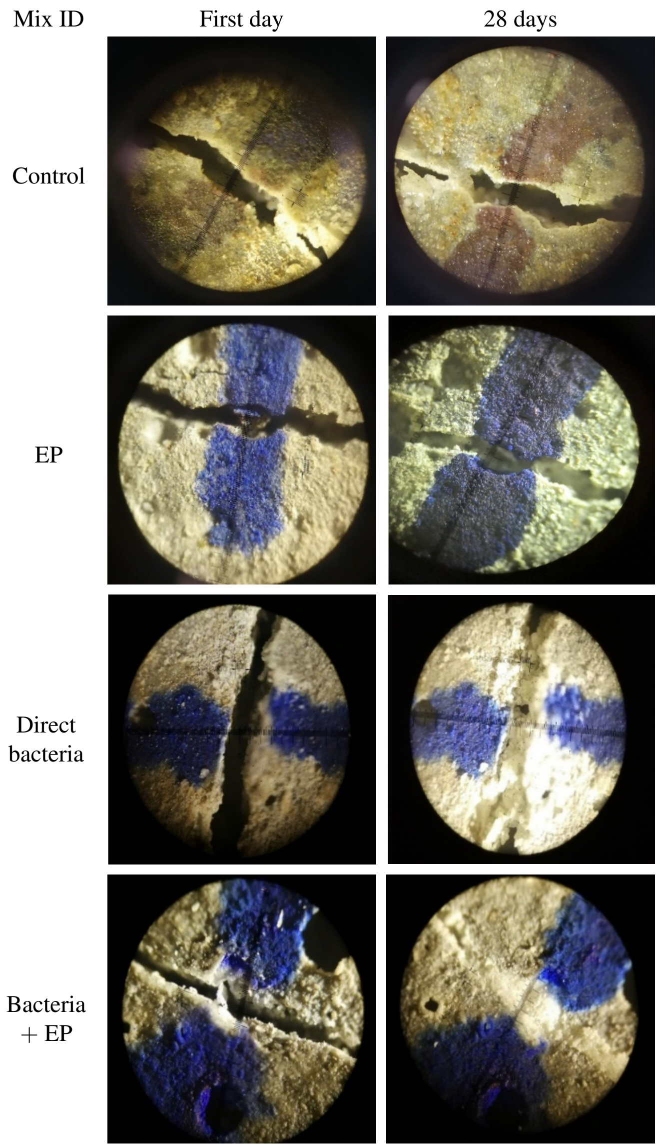

Fig. 6. Visual inspection of self-healing

Cracks produced in the control specimens did not show noticeable healing, despite the fact that calcium lactate pentahydrate and the process of autogenous healing resulted in very small-scale healing of tiny cracks, whereas some cracks were repaired as a result of abundant mineral precipitation on the cracked region in all EP induced microbial formulations, confirming that bacteria immobilized in EP are capable of producing large amounts of healing product as shown in Fig. 7.

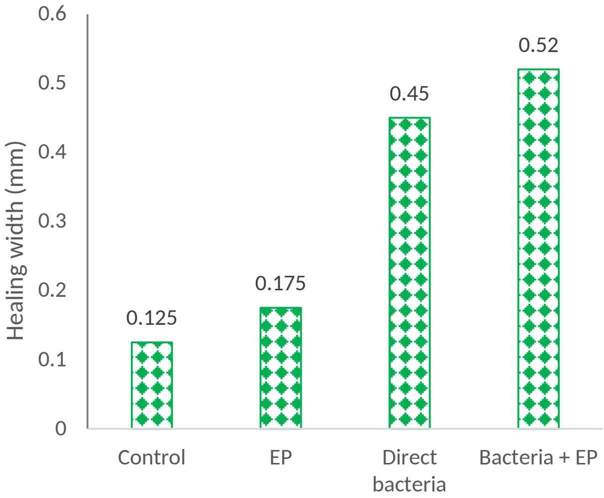

Fig. 7. Healing width

In all EP-reinforced microbial specimens, substantial healing was observed under a crack-width measuring microscope, especially after 28 days of exposure to healing conditions. Organic nutrition is abundant in the concrete mix during the initial days of healing, which is devoured by bacteria to generate $CaCO_3$. Healing was measured in terms of the difference in crack width before healing and its width after healing. Previous studies that used EP as a bacterium carrier found healing of up to 0.78 mm [18].

This project resulted in the total healing of fissures up to 0.78 mm in diameter. Because hydration is not complete at this age, additional hydration of cement was used to seal some fissures [35]. As a result, the healing accomplished in concrete samples that were cracked at the 28-day intervals was lower than in the samples pre-cracked at 7 days. The healing parameters in the other groups of specimens did not change appreciably over time. Only 0.2 mm of crack closure was achieved in the control specimen. Worth-considering fact is that the results of visual observation are not viable enough to be referenced to internal fracture healing because only a few cracks were identified, and they were difficult to discern over time.

3.3. Digital image processing

The authors used digital image processing to measure the crack diameters of concrete. The crack area to pixel count is measured before and after the healing by using digital image processing in MATLAB software according to a past study [36]. The original image is imported into the workspace by setting variables. The original image is also referred to as an RGB image. Then the RGB image is converted into a grey-scale image. Threshold valuing is the simplest method of converting a grey-scale image to a binary image as shown in Fig. 8. MATLAB software was used to calculate the extent of healing in terms of the proportion of crack pixels replaced by $CaCO_3$ produced by bacteria. The pixel count of concrete different blends is shown in Fig. 9. It can be observed that pixel count after healing decreases with EP and direct bacteria. However, a maximum decrease was observed with a combination of EP with bacteria. Therefore, the study recommends using EP in combination with bacteria instead of direct bacteria.

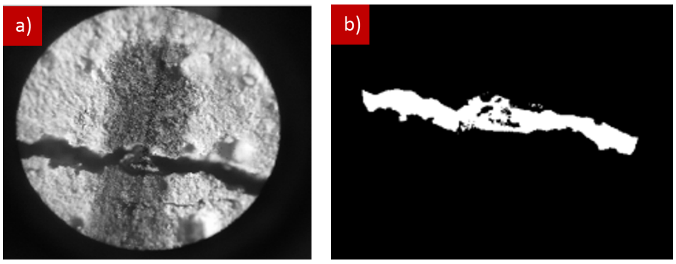

Fig. 8. (a) Greyscale image, (b) binary image

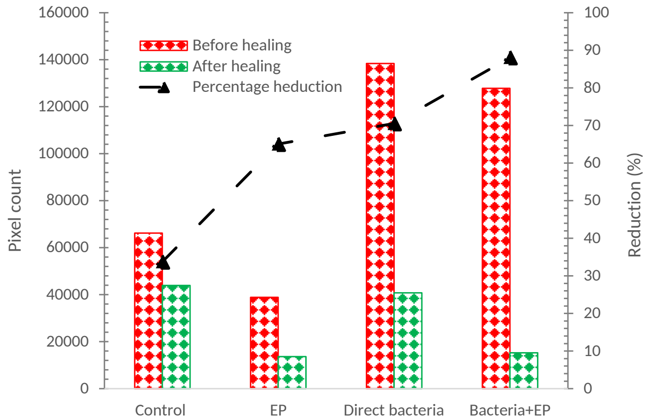

Fig. 9. Pixels in the crack before and after healing

4. CONCLUSIONS

The study examined the suitability of using expanded perlite as a protective carrier for the immobilization of bacteria spores in concrete for self-healing. The potential usage of *Sporosarcina aquimarina* – NCCP-2716 as a microbial agent for the self-healing of concrete was investigated. After a certain number of days of healing, the compressive strength, strength regain, and variations in fracture widths were studied. Digital image processing was also performed. The following findings were drawn from this investigation based on the gathered and evaluated results.

- The compressive strength of concrete slightly decreased with the addition of EP, i.e. 7.6% less than from the reference mix (concrete without EP). However, in the case of combined addition (EP and bacteria), the compressive strength is approximately equal to the reference concrete.
- Bacterial spores are immobilized in expanded perlite successfully conserved calcium lactate pentahydrate and bacterial metabolic activity, resulting in enough $CaCO_3$ generation.
- Under controlled settings, 0.8 mm crack healing was achieved after the 28-day healing period in the samples that were pre-cracked after 7 days. The ability of bacteria immobilized in expanded perlite to perform crack repair in concrete was proven by visual observation using a crack-detecting microscope.

- EP provided stronger protection to germs as a carrier, as seen by its crack-healing and pore-filling abilities.
- The sample showed a considerable self-healing phenomenon caused by substantial calcite precipitation by bacteria. The induced cracks were detected to be repaired autonomously by the calcite produced by the bacteria without any harmful effect on strength. The findings of this study may serve as a scientific basis for the usage of expanded perlite as a novel microbe carrier and *Sporosarcina aquimarina* as a prospective microbe in bacteria-based self-healing concrete.

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